

# Dynamics of deep soil water, organic carbon and total nitrogen in response to the conversion of annual crops to long-term alfalfa pasture on the semi-arid Loess Plateau

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## Research Article

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# Abstract

*Aims* Deep soil resources was not well quantified under dryland conditions. This study aimed to quantify the changes of deep soil water, soil organic carbon (SOC), and total nitrogen (TN) in alfalfa pasture, and identify the relationships between root density and their changes.

*Methods* Field experiment was conducted in the Loess Plateau in 2020 and 2021. Soil water, SOC, and TN contents and alfalfa root density to 1000-cm-depth were measured in alfalfa pastures and an annual crop reference field.

*Results* Soil water depletion by alfalfa mainly occurred in the first six years with a depletion rate of 41.6, 49.1, and 62.1 mm year<sup>-1</sup> in the shallow (0-200 cm), middle (200-500 cm), and deep (500-1000 cm) soil layers, respectively. Total depletion after six and 19 years were 916.8 and 1049.4 mm respectively. SOC content in all layers increased and peaked six years after planting, with total storage in the 0-1000-cm-profile of 74.6 kg m<sup>-2</sup>, 43.3% higher than in the reference field. TN storage in only the shallow layer continuously increased and was 52.5% higher than that in the reference field after 19 years. Root density explained the changes of soil water in the shallow and deep soil layer, SOC in the shallow and middle layer, and TN only in the shallow soil layer.

*Conclusions* Although alfalfa pasture continuously reduced deep soil water, it showed great potential for soil carbon and nitrogen sequestration. It should be stopped early ( $\leq 6$  years) to increase water sustainability and maintain carbon and nitrogen sequestration efficiencies.

## Introduction

The Chinese Loess Plateau (CLP) is in the upper and middle reaches of the Yellow River with an area of 64×10<sup>4</sup> km<sup>2</sup> (34°-41°N, 98°-114°E), there are large reserves of arable land, and it is one of the most important farming areas in China (Wang et al., 2009; Ren et al., 2011). The region has a semiarid monsoon climate, water resources are limited and yields of the traditional grain crop production system are unstable (Wang et al., 2020). In addition, this area is characterized by deep loess deposits and has a fragile ecosystem that is vulnerable to soil erosion (Li and Huang, 2008; Fu et al., 2016). The Chinese government issued a series of environmental policies in the 1990s that promoted the establishment of pasture and shrubland through the conversion of cropland on hill slopes to reduce soil erosion. Alfalfa (*Medicago sativa* L.), a deep-rooted perennial legume crop, has high canopy coverage and pasture yields, which was widely planted in the ecological rehabilitation project (Yuan et al., 2016). In addition, the price of alfalfa hay increased greatly in recent years; more and more croplands were converted to alfalfa pasture to increase the output of the farmland.

Introducing perennial pastures into farmland and redesigning the planting structures in a farming system should consider soil water dynamics to maintain the sustainable development of the environment, particularly in arid and semiarid regions (Beate and Haberlandt, 2002; Vereecken et al., 2014; Oldroyd and Dixon, 2014). Alfalfa is a summer-active forage crop with a high evapotranspiration rate and deep rooting

system that provides it access to water deeper in the soil than can be accessed by annual crops (Liu and Shao, 2015; Huang et al., 2018). Research in Australia has shown that alfalfa can use at least 50 mm more water than annual pastures or crops (Ridley et al., 2001), creating a larger soil water deficit, leading to potentially less groundwater recharge (Macfarlane, 1995). In the Loess Plateau, Li (1983) firstly observed that growing alfalfa continuously for six years produces a relatively dry layer in the deep soil profile from 200 to 1000 cm, indicating the potential adverse effects of the dry layer on regional hydrological processes. Li and Huang (2008) found alfalfa to decrease soil water storage in the 0-500 cm profile at a rate of 33.5 mm year<sup>-1</sup> and suggested that the length of the alfalfa phase in rotation should be less than eight years to restore soil water deficit in farmland. A regional synthesis study in CLP had shown that alfalfa soil water content (SWC) decreased by 14.3%, 27.0%, and 35.6% in the 0-5-, 5-10- and > 10-year-old alfalfa fields, respectively (Ali et al., 2021). However, long-term experiment was rarely conducted. How the soil water deficit in different layers developed with the planting ages of alfalfa is still unclear, and how the adverse soil water environment in deep loess affects the carbon and nitrogen sequestration has not yet been clarified.

Soil organic carbon (SOC) and total nitrogen (TN) storage play an essential role in sustainable agriculture. Converting forest land and grassland to arable land is known to decrease the content of SOC and TN whereas converting land under annual crops into perennial grasslands can increase carbon and nitrogen sequestration (Su et al., 2007; Zhang et al., 2009). Land reclamation in the 1980 and 1990s on the CLP caused severe soil erosion. Fu et al. (2010) suggested that reducing the SOC and TN by decreasing native forestry or pasture can be slowed by improving soil management, such as increasing the use of no-till and converting croplands to forestry or forestry pasture. Alfalfa pasture can fix nitrogen and increase soil fertility, and sequester carbon dioxide from the atmosphere into deep soil layers (Jiang et al., 2006). In the north of Culbertson in the USA, Sainju (2011) found that the SOC and TN in 0–15 cm soil depth was greater in continuous alfalfa grassland than those in durum-barley (*Hordeum vulgare* L.) hay and durum-foxtail millet (*Setaria italica* L.) hayfield. Research in the middle of the Hexi Corridor region of China indicated that converting vegetable land to alfalfa land with low nitrogen application could significantly increase SOC and TN (Yu et al., 2020). Chen et al. (2017) found that SOC and TN under alfalfa fields in the CLP were 10% and 16% higher than cropland in 0-100 cm soil depth. Song et al. (2021) also found that the SOC content under the 17-yr-old alfalfa fields in the 0–20 and 20–60 cm layers in the Loess Plateau, with average values of 12.38 and 9.77 g kg<sup>-1</sup>, respectively, were significantly higher than those of fallow land. Most of the above studies focused on the carbon and nitrogen in the surface soil layers, how the SOC and TN contents in the deep soil layers change with the planting age of alfalfa, and soil water conditions are not well studied. This information is essential for farming system design under the background of reducing chemical fertilizer application and trying to achieve carbon neutrality. In addition, previous studies showed that the consequence of the long-term soil carbon sequestration is at the cost of water depletion and soil desiccation on the CLP (Zhang and Shanguan, 2016; Lan et al., 2021). The relationships between soil water depletion and soil carbon and nitrogen stock changes in alfalfa fields are unclear. There should be a way to increase carbon and

nitrogen stock and reduce fertilizer use while not affecting water sustainability by properly using alfalfa in farming system design.

Therefore, this study was conducted a) to quantify the effect of the long-term planting of alfalfa on SWC in the 0-1000 cm profile and describe the dynamics of soil water storage depletion rate by alfalfa in different soil depths; b) to analyse the distribution of SOC and TN contents in the 0-1000 cm profile and describe the dynamics of SOC and TN storage increment in different soil layers after planting alfalfa; and c) to demonstrate the relationships between soil water depletion and SOC and TN storage increment. The results can help guide the efficient and sustainable management of alfalfa pasture and annual crop rotation practices in the CLP and in other similar regions.

## Materials And Methods

### Site description

The CLP region is in the upper and middle reaches of the Yellow River and covers arid and semiarid temperate climate zones in the north and south, respectively. The spatial distribution of annual precipitation is quite variable, with a mean level of 421.8 mm (Fig. S1). The yearly distribution of the rainfall is also uneven, with approximately 60% occurring during the summer and autumn months. The mean regional annual temperature is 9.0°C (Zhang et al., 2014). The plateau's altitude ranges between 1000–1600 m above sea level. The surface is covered by highly erodible loess layers approximately 100 m thick on average (Xin et al., 2011).

This study was carried out at the Qingyang Experimental Station of Lanzhou University, which is located in the south of CLP (35°77'N, 107°51'E, and altitude 1297 m, Fig. S1). It has a semi-arid continental monsoon climate, with an average annual temperature of 10.1°C and average precipitation of 567.9 mm in recent 20 years (2001 – 2020). Rainfall occurred from June to September accounting for 63.4% of the yearly total. The soil at the site is Heilu soil (the Los-Orthic Entisols based on the FAO soil classification). The soil has weak cohesion and high water retention, and also the soil is deep and prone to erosion. Selected soil physical and chemical properties in the 0–1000 cm soil layer are shown in table S1.

### Experiment design

Four pieces of alfalfa fields in the experiment station were selected for testing. Samples were taken in July 2020 under 3-, 6-, 11-, and 18-year-old alfalfa fields, and in July 2021 under 4-, 7-, 12-, and 19-year-old alfalfa fields. All fields have the same variety of “Longdong”, a widely used local variety, and they were managed in similar measures according to local practices. Alfalfa was planted in August after harvesting winter wheat in early July. Initial nitrogen fertilization of 80 kg ha<sup>-1</sup> and P<sub>2</sub>O<sub>5</sub> fertilization of 120 kg ha<sup>-1</sup> were applied before the planting, and no more fertilizer was applied during the growth cycle of alfalfa. Two cuttings were conducted in the first year after planting, and three cutting were conducted in approximately late May, mid-July, and late September in the following years. An annual crops field in the experiment station was selected as a reference field; the planting history was winter wheat and maize

rotation. Therefore, the experiment comprised nine treatments, namely, 3-, 4-, 6-, 7-, 11-, 12-, 18-, and 19-year-old alfalfa fields (M3, M4, M6, M7, M11, M12, M18, and M19) and the reference field (RF).

## Sampling and measurements

In 2020 and 2021, alfalfa was cut three times on May 30, July 15, and September 25, respectively. After each sampling, the alfalfa biomass yield was measured after drying in an oven at 65°C to constant weight. The annual dry matter yield of alfalfa was the total amount of biomass sampled from three cuts.

Four replicate sampling points were selected in each field. The soil was sampled using a hand augur (inner diameter of 80 mm) at depth intervals of 10 cm to 1000 cm. Subsamples were taken from soil samples every 40-cm-depth to measure SWC; 25 subsamples were thus measured for each replicate. Soil mass water content was measured by the oven-drying method and converted to volumetric SWC by multiplying the bulk density value. Soil water storage (SWS, mm) and soil water deficit (SWD, mm) in soil layer  $i$  were calculated as:

$$SWS_i = \sum_{i=1}^n SWC_i \times H_i \times 10$$

1

$$SWD_i = SWS_{i,A} - SWS_{i,R}$$

2

where,  $SWC_i$  (%) was soil water content in layer  $i$ ,  $H_i$  (cm) was height of soil layer  $i$ ,  $SWS_{i,A}$  mm and  $SWS_{i,R}$  are soil water storages in layer  $i$  in alfalfa and reference fields respectively.

When measuring SOC (g kg<sup>-1</sup>) and TN (mg kg<sup>-1</sup>) contents, soil samples were classified to 0–10, 10–30, 30–60, 60–100 cm in the 0-100 cm layer, and was classified every 100 cm in depth in the 100–1000 cm layer, 13 soil subsamples were thus measured for each replicate. The soil was further passed through a 0.25-mm sieve for the determination of SOC content. The SOC content was measured using a modified Walkley and Black method (Walkley & Black, 1934). We extracted 0.5 g soil samples with 5 mL of 0.8 mol L<sup>-1</sup> K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and 10 mL of concentrated H<sub>2</sub>SO<sub>4</sub> at 150°C for 30 min, then titrated the extracts with 0.2 mol L<sup>-1</sup> FeSO<sub>4</sub>. The soil TN content was measured via the Kjeldahl procedure (Foss Kjelttec 8400, FOSS, DK). Blanks were included in each analysis to detect possible sources of contamination. SOC storage (SOCS, kg C m<sup>-2</sup>) and TN storage (TNS, kg N m<sup>-2</sup>) in soil layer  $i$  were calculated as:

$$SOCS = 0.01 \times SOC_i \times B_i \times H_i$$

3

$$TNS = 0.01 \times TN_i \times B_i \times H_i$$

4

where,  $BD_i$  ( $\text{g cm}^{-3}$ ) is soil bulk density in layer  $i$  and 0.01 is a conversion coefficient.

The water depletion efficiency for SOC accumulation ( $WDE_{\text{SOC}}$ ) and TN accumulation ( $WDE_{\text{TN}}$ ) were defined to quantify how much SOC and TN were accumulated by per mm of water depletion:

$$WDE_{\text{SOC}} = \frac{SOCS_A - SOCS_R}{SWD}$$

5

$$WDE_{\text{TN}} = \frac{TNS_A - TNS_R}{SWD}$$

6

where,  $SOCS_A$  and  $SOCS_R$  are soil organic carbon storages in alfalfa and reference fields, respectively; and  $TNS_A$  and  $TNS_R$  are soil total nitrogen storages in alfalfa and reference fields, respectively.

The soil cores were put into nylon mesh bags and then washed carefully with tap water to remove all the soil. The roots were scanned using a EPSON Scan (Expression 11000xl, Canada), and then the root parameters were determined using WinRhizo software (version 5.0 Regent Instruments Inc., Quebec, Canada) based on the images showing fine roots. After the roots were scanned, the fine roots (< 2 mm diameter) length density (FRLD,  $\text{cm cm}^{-3}$ ) was calculated as follows:

$$\text{FRLD} = \frac{\text{FRL}}{V_s}$$

7

Where FRL is fine root length (cm); and  $V_s$  is the volume of sampling soil ( $\text{cm}^{-3}$ ).

## Date analysis and statistics

Statistical analyses were performed using the SPSS 25.0 software package (SPSS Inc., Chicago, IL, USA). The measured data of soil water, SOC and TN contents were tested for normality and homogeneity, and logarithmic transformations were performed when necessary. Soil was separated into shallow (0-200 cm), middle (200–500 cm), and deep (500–1000 cm) layers to simplify the analyses. A two-way analysis of variance was used to analyze the effects of planting age and soil layer on SOC and TN storage. If a significant difference ( $P < 0.05$ ) was detected among treatments, means were compared using Duncan's test. Associations between variables were assessed by linear and non-linear regression and correlation analysis.

## Results

### SWC and soil water storage depletion

Figure 1 shows the distribution of SWC in the 0-1000 cm soil profile in different aged alfalfa fields compared with that in RF. SWC in RF varied from approximately 20%-25%, with a mean value of 21.7%. SWC in the profile of M3 was substantially lower than that of RF except for those values below 800-cm-depth, averaged at 18.8%. The SWC of M4 was lower than that of M3 in the 0-300 and 500–1000 cm soil layers, with a mean value of 17.0%. After six years of planting (M6), SWC in 0-400 cm nearly decreased to the permanent wilting point; the mean value in the 0-1000 cm profile was 14.6%, 32.5% lower than that in RF. From 6 to 19 years after planting, SWC showed few changes throughout the whole profile.

Figure 2 shows the amount of soil water storage depletion by alfalfa in the shallow (0-200 cm), middle (200–500 cm), and deep (500–1000 cm) layers as changed with the age of alfalfa. Soil water depletion was 144.8 and 149.5 mm in the shallow and middle layers in M3, respectively, and was only 85.2 mm in the deep layer. The depletion increased substantially from three to six years after planting, reaching 249.5, 275.8, and 391.5 mm in the shallow, middle, and deep layers, respectively, in the 6-year-old alfalfa field. However, the depletion rarely increased after six years of planting, especially in the shallow and middle layers. The total amount of soil water depletion in 0-1000 cm profile were 373.3, 610.5, 916.8, 959.1, 966.4, 985.0, 1038.7, and 1049.4 mm in M3, M4, M6, M7, M11, M12, M18, and M19, respectively. Exponential equations well fitted the relationships between soil water storage depletion and the age of alfalfa ( $R^2 > 0.95$ ,  $P < 0.001$ ).

### SOC content and SOC storage increment

Figure 3 shows the distribution of SOC content in the 0 – 1000 cm soil profile in different aged alfalfa fields compared with that in RF. SOC content exponentially decreased with the soil depth in the 0-200 cm layer and maintained relatively stable values in the below layers. RF had the lowest values throughout the whole profile, while M6 maintained the highest. After three years of planting, the SOC contents throughout the 0-800 cm soil profile significantly increased and continued until six years of planting. Compared with M6, the SOC contents under M7 decreased and showed few further changes after seven years of planting. Averaged over the whole profile, the SOC content in RF, M3, M4, M6, M7, M11, M12, M18, and M19 were 4.7, 5.9, 6.2, 6.8, 6.2, 6.1, 6.3, 6.1 and 6.2  $\text{g kg}^{-1}$ , respectively.

Figure 4 shows SOC storage in the shallow (0-200 cm), middle (200–500 cm), and deep (500–1000 cm) layers in different aged alfalfa fields and RF. Both alfalfa planting age and soil layer showed significant effects on SOC storage ( $P < 0.001$ ), while their interaction had no significant effect ( $P > 0.05$ ). SOC storage under alfalfa fields was significantly higher than that under RF for all soil layers. M6 and RF had the highest and lowest values, respectively. The total amount of SOC storage in 0-1000 cm was as high as 74.6  $\text{kg m}^{-2}$  in M6, 43.3% higher than that in RF.

Figure 5 shows the SOC storage increment in different aged alfalfa fields than RF. The increment increased substantially from three to six years after planting, reaching the maximum value of 7.0, 6.1, and 9.5 kg m<sup>-2</sup> in the shallow, middle, and deep soil layers, respectively, in the 6-year-old alfalfa field. The increment decreased from six to eleven years after planting and maintained stable values after that. The Gaussian functions well fitted the relationships between SOC increment and alfalfa planting age ( $R^2 > 0.80$ ,  $P < 0.05$ ).

## **TN content and TN storage increment**

Table 1 lists the soil TN content in the 0-1000 cm profile in different treatments. Soil TN content decreased with soil layers, that in the shallow 0-100 cm layer was much higher than below layers, with values all greater than 0.5 g kg<sup>-1</sup>. The TN content in the 0-200 cm layer was significantly increased after planting alfalfa, and it continuously increased with the planting age of alfalfa. The highest values were found in M18, M19, M12, M12, and M19 in 0–10, 10–30, 30–60, 60–100, and 100–200 cm layers, respectively. There was no significant difference in soil TN content among different treatments below the 200-cm-depth.

Table 1

The contents of total nitrogen ( $\text{g kg}^{-1}$ ) in the 0 – 1000 cm soil profile under different treatments. RF refers to annual crop field and M3, M4, M6, M7, M11, M12, M18, and M19 represent 3-, 4-, 6-, 7-, 11-, 12-, 18-, and 19-year-old alfalfa fields, respectively. Values are means  $\pm$  standard deviations.

Soil layer (cm)	RF	M3	M4	M6	M7	M11	M12	M18	M19	P value
0–10	0.64 $\pm$ 0.14	1.09 $\pm$ 0.02	1.27 $\pm$ 0.14	1.31 $\pm$ 0.13	1.53 $\pm$ 0.12	1.69 $\pm$ 0.14	1.43 $\pm$ 0.04	1.97 $\pm$ 0.27	1.90 $\pm$ 0.05	P < 0.001
10–30	0.71 $\pm$ 0.21	0.93 $\pm$ 0.10	1.01 $\pm$ 0.05	0.94 $\pm$ 0.06	1.08 $\pm$ 0.06	1.08 $\pm$ 0.09	1.07 $\pm$ 0.09	1.10 $\pm$ 0.16	1.16 $\pm$ 0.04	P < 0.05
30–60	0.49 $\pm$ 0.05	0.61 $\pm$ 0.12	0.72 $\pm$ 0.03	0.73 $\pm$ 0.04	0.70 $\pm$ 0.05	0.76 $\pm$ 0.08	0.78 $\pm$ 0.06	0.72 $\pm$ 0.03	0.75 $\pm$ 0.12	P < 0.05
60–100	0.52 $\pm$ 0.11	0.66 $\pm$ 0.06	0.64 $\pm$ 0.05	0.67 $\pm$ 0.05	0.64 $\pm$ 0.08	0.65 $\pm$ 0.07	0.68 $\pm$ 0.07	0.64 $\pm$ 0.10	0.65 $\pm$ 0.13	P < 0.001
100–200	0.33 $\pm$ 0.12	0.38 $\pm$ 0.07	0.44 $\pm$ 0.07	0.42 $\pm$ 0.11	0.45 $\pm$ 0.08	0.45 $\pm$ 0.08	0.43 $\pm$ 0.03	0.43 $\pm$ 0.01	0.48 $\pm$ 0.10	P < 0.05
200–300	0.30 $\pm$ 0.07	0.33 $\pm$ 0.04	0.30 $\pm$ 0.09	0.27 $\pm$ 0.05	0.28 $\pm$ 0.07	0.26 $\pm$ 0.03	0.30 $\pm$ 0.08	0.26 $\pm$ 0.09	0.29 $\pm$ 0.07	NS
300–400	0.27 $\pm$ 0.06	0.30 $\pm$ 0.02	0.28 $\pm$ 0.07	0.29 $\pm$ 0.05	0.28 $\pm$ 0.06	0.29 $\pm$ 0.04	0.28 $\pm$ 0.02	0.26 $\pm$ 0.05	0.30 $\pm$ 0.02	NS
400–500	0.28 $\pm$ 0.01	0.31 $\pm$ 0.01	0.33 $\pm$ 0.02	0.37 $\pm$ 0.02	0.35 $\pm$ 0.02	0.35 $\pm$ 0.01	0.34 $\pm$ 0.05	0.29 $\pm$ 0.08	0.33 $\pm$ 0.04	NS
500–600	0.31 $\pm$ 0.07	0.31 $\pm$ 0.01	0.32 $\pm$ 0.01	0.34 $\pm$ 0.04	0.37 $\pm$ 0.03	0.34 $\pm$ 0.02	0.36 $\pm$ 0.05	0.31 $\pm$ 0.05	0.32 $\pm$ 0.03	NS
600–700	0.31 $\pm$ 0.01	0.32 $\pm$ 0.02	0.31 $\pm$ 0.02	0.33 $\pm$ 0.03	0.35 $\pm$ 0.01	0.33 $\pm$ 0.06	0.37 $\pm$ 0.03	0.31 $\pm$ 0.06	0.34 $\pm$ 0.03	NS
700–800	0.31 $\pm$ 0.06	0.31 $\pm$ 0.02	0.33 $\pm$ 0.01	0.31 $\pm$ 0.04	0.34 $\pm$ 0.06	0.34 $\pm$ 0.03	0.33 $\pm$ 0.04	0.29 $\pm$ 0.04	0.32 $\pm$ 0.02	NS
800–900	0.25 $\pm$ 0.10	0.27 $\pm$ 0.02	0.27 $\pm$ 0.06	0.25 $\pm$ 0.04	0.26 $\pm$ 0.03	0.25 $\pm$ 0.02	0.27 $\pm$ 0.03	0.23 $\pm$ 0.04	0.27 $\pm$ 0.03	NS

Soil layer (cm)	RF	M3	M4	M6	M7	M11	M12	M18	M19	P value
900–1000	0.24 ± 0.01	0.24 ± 0.03	0.23 ± 0.06	0.26 ± 0.03	0.28 ± 0.02	0.23 ± 0.01	0.26 ± 0.02	0.23 ± 0.01	0.23 ± 0.02	NS

Figure 6 shows soil TN storage in the shallow (0-200 cm), middle (200–500 cm), and deep (500–1000 cm) layers in different treatments. TN storage in the shallow layer differed a lot among treatments, with M19 had the largest value of  $1.83 \text{ kg m}^{-2}$  and RF had the lowest value of  $1.20 \text{ kg m}^{-2}$ . TN storage was not significantly increased with the age of alfalfa in the below layers. Summed over the 0-1000 cm soil profile, TN storage were 4.14, 4.58, 4.67, 4.68, 4.96, 4.86, 4.79 4.76 and  $4.95 \text{ kg m}^{-2}$  in RF, M3, M4, M6, M7, M11, M12, M18, and M19, respectively.

Figure 7 shows the dynamics of soil TN increment after the planting of alfalfa. We can see that TN increment was exponentially increased with the age of alfalfa in the shallow soil layer ( $P < 0.05$ ); however, in the middle and deep soil layers the increment was rarely changed, the regression showed insignificant changing tendencies ( $P > 0.05$ ).

## Root distribution and the relationships with SWD, SOC and TN

The distribution of FRLD in the 0-1000 cm soil profile under the different aged alfalfa fields is shown in Fig. 8. The FRLD had a similar vertical pattern under all treatments, decreasing exponentially in the shallow 200 cm soil depth and maintaining stable values below in the below layers. Specifically, alfalfa roots in M3 are distributed within the 0-800cm soil depth, and after four year's planting, the roots are distributed to the 1000-cm-depth. The FRLD in the 0-200 cm soil layer in M3 was significantly lower than that in M4, while that in the below layers was not significantly different (Fig. 8a). Also, no apparent difference in FRLD was found between M6 and M7, M11 and M12, and M18 and M19, except in 0–10 cm between M6 and M7.

Figure 9 compares the average FRLD in the shallow (0-200 cm), middle (200–500 cm), and deep (500–1000 cm) layers among different treatments. The FRLD in the shallow layer was much higher than in the below layers. In every soil layer, the FRLD largely increased from three to six years after planting alfalfa and gradually decreased after that (in shallow and middle layers) or maintained a stable value (in deep layer).

Figure 10 shows the linear regressions of FRLD with soil water depletion, SOC storage increment, and TN storage increment. In the in shallow soil layer, all the regressions were significantly positive, root density well explained the soil water depletion and SOC and TN increments. However, in the below layers, the regressions were only significant for SOC storage increment in middle layer and for soil water depletion in the deep layer. When the regression was made separately for different aged alfalfa, we found that SOC

storage increment in deep soil was also significantly positively related with FRLD in 3, 4, and 6 years old alfalfa fields (Fig. S2).

## Discussion

### Soil water depletion

Deep-rooted plants will take up deep soil water when water in shallow soil water availability is limited (Zhang et al., 2020). Over depletion of deep soil water would form a dried soil layer, described as the soil layer with a water content range between the permanent wilting point and the stable field capacity (Li, 1983). Previous studies on the CLP have mainly discussed the dried soil layer caused by afforestation (Deng et al., 2016; Su and Shanguan, 2019). We focused on alfalfa, the most widely planted pasture on the CLP, for environmental conservation and farming system diversification. We found that soil water depletion in the shallow (0-200 cm in-depth), middle (200–500 cm in-depth), and deep (500–1000 cm in-depth) soil layers in the alfalfa field was as high as 262.0, 317.3, and 470.1 mm, respectively, after 19 years of planting, with the total amount of water depletion of 1049.4 mm. The value was comparable with the depletion in 200–1000 cm layer of 844 mm in a 16-year-old alfalfa field reported by Li and Huang (2008). The total depletion was also comparable with the values of 1139.6-1169.3 mm that occurred in a 36-year-old artificial forest in the north CLP (Lan et al., 2021), and the value of 1106 mm reported in a 20-year-old apple orchard (Zhang et al., 2017), indicating that the perennial pasture or tree species depleted soil water to the approximately permanent wilting point after long-term of planting.

Besides, we described the dynamic of water depletion as changing with the planting age of alfalfa in this work. The depletion was mainly occurred during the first six years after planting with a depletion rate of 41.6, 49.1, and 62.1 mm year<sup>-1</sup> in the 0-200, 200–500, and 500–1000 cm soil layers, respectively, while the corresponding rate from 6–19 years after planting were only 1.0, 1.7, and 7.5 mm year<sup>-1</sup>. We found exponential equations could well fit the relationships between soil water storage depletion and the age of alfalfa in different soil layers. A field experiment conducted in north CLP showed a similar decreasing pattern; soil water storage in alfalfa field decreased approximately 300 mm after three years of planting and slowly decreased by only 100 mm from 3–15 years after planting (Jia et al., 2009).

Alfalfa returns green early in the spring and is harvested twice a year in the north CLP and three times in the central and south CLP. The high soil water depletion rate of alfalfa was mainly due to the high coverage and annual long coverage time compared to annual crops (Liu and Shao, 2015). Alfalfa extends its roots in deep soil in the early ages to deplete soil water and sustain a high production under rain-fed conditions. We validated that the alfalfa root system was distributed in the entire 0-1000 cm soil layer after four years of planting. Water depletion in the deep soil layer showed a significant positive relationship with FRLD, indicating that the root system absorbed deep soil moisture to sustain alfalfa production. When deep soil availability decreased after several years of planting, water use and yield of alfalfa would gradually decrease (Li and Huang, 2008; Ren et al., 2011; Gu et al., 2018). We found that

yield of alfalfa biomass decreased after six years of planting (Fig. S3), which paralleled well with the dynamic of deep soil water content.

## **SOC and TN storage increment**

The establishment of either forest or pasture on degraded cropland has been proposed as an effective method for climate change mitigation because these land-use types can increase SOC stocks (Ashagrie et al., 2007; Deng et al., 2014). This study investigated the dynamic of SOC in the deep soil layer and found that the SOC contents in the whole 0-1000 cm soil profile all increased after the annual crop field converting to alfalfa pasture. The SOC increment was 43.3% higher in the six-year-old alfalfa field than in the annual crop field, indicating the excellent carbon sequestration potential of planting alfalfa on the deep loess. Averaged over different aged fields, SOC storage in the 200–1000 cm layer accounted for 70.1% of the total storage in the 0-1000 cm layer. Therefore, it is also indicated that alfalfa fields store large amounts of SOC below the topsoil profile. Soil carbon storage might have been greatly underestimated in this area and other similar regions if only soil carbon in the shallow layers were considered.

SOC was mainly affected by the input, decomposition, and transformation of organic matter in different soil layers (Rumpel and Kögel-Knabner, 2011). The residue of annual crops was commonly removed from the field, whereas that of alfalfa would not be harvested after the last cut and covered the field throughout the winter (Wang et al., 2020). On the other hand, alfalfa pasture has many roots in the shallow soil layer (Fig. 8), which are essential for soil organic matter formation. Therefore, the higher SOC content in the 0-200 cm in alfalfa fields mainly resulted from both above- and below-ground biomass input into the soil. Additionally, Mu et al. (2014) indicated that the addition of nitrogen derived from nitrogen-fixing legume species could lower soil carbon decomposition, which might be another important reason for surface soil organic carbon accumulation. In middle and deep soil layers (200–1000 cm in-depth), the formation of SOC is mainly affected by root distribution and activities (Lan et al., 2021). Initially, organic matter from roots contributes to carbon's coarse and light fractions. Under long-term stable conditions, carbon is transferred slowly from the light fractions to the heavy or fine fractions (Jiang et al., 2006). From the life cycle point of view, we also found that the SOC storage in the alfalfa field peaked at the planting age of six-year-old. This might be because deep soil water was depleted and the reduced above- and below-ground alfalfa biomass production limited soil organic matter formation. Meanwhile, the alfalfa roots remained active in the middle and late ages (Fig. 8) and increased the decomposition of soil organic matter (Li et al., 2019).

In general, soils in pasture-based cropping systems contain a higher proportion of N fractions and can therefore generate savings of N fertilizer (Jiang et al., 2006). Alfalfa can derive N from symbiotic fixation, which was expected to have a synergetic positive effect on soil N. We found TN storage in the top 200 cm soil layer steadily increased with the planting age of alfalfa. However, in the below layers (200–1000 cm), soil N content was not significantly different among different aged alfalfa fields, soil N was less affected by existence of alfalfa than SOC. Soil nutrients balance is governed by the quantities of fertilizer added to soil and those of nutrients uptake by plants. We found that soil TN was positively related to FRLD in the

0-200 cm layer (Fig. 10), massive root system contributed to the soil TN accumulation. Therefore, soil TN in the shallow layers resulted from both biological N<sub>2</sub> fixation, larger litter input, and fine root biomass decomposition (Zhang et al., 2009; Li et al., 2019). However, decomposition of root biomass was the primary nitrogen source in the middle and deep soil layers (200–1000 cm), which might be balanced by plant uptake to support plant growth. Rotating alfalfa pasture with annual grain crops would be an efficient way to save fertilizer and improve farming system sustainability.

## Water depletion efficiency for SOC and TN increment

In the arid and semiarid areas, the consequence of the long-term soil carbon sequestration in artificial forests and pastures is at the cost of water depletion and soil desiccation (Li et al., 2019). To quantify the trade-off between soil carbon and nitrogen accumulation and soil water consumption in the alfalfa field, we made regressions between soil water depletion and SOC and TN storage increment (Fig. S4). Large amounts of carbon accumulation in early ages contributed to the high water-depletion efficiency, with values of 30.2, 26.2, 24.6, and 16.5 g m<sup>-2</sup> SOC increment mm<sup>-1</sup> water depletion for 3-, 4-, 6-, and 7-year old alfalfa fields. The efficiency decreased with the alfalfa planting age. At 19 years after planting, alfalfa consumed 1049.4 mm soil water storage in the 0-1000 cm profile and increased SOC storage by 13.1 kg m<sup>-2</sup>, with the average water depletion efficiency of 12.4 g m<sup>-2</sup> SOC increment mm<sup>-1</sup> water depletion. The efficiency is significantly higher than the value of 0.81 g m<sup>-2</sup> SOC increment mm<sup>-1</sup> water depletion reported by Lan et al. (2021) for forest fields, as they detected only 0.94 kg m<sup>-2</sup> SOC increment. In comparison, the efficiency for nitrogen accumulation linearly increased with the planting age of alfalfa in the 0-200 cm layer and slightly decreased in the 200–500 and 500–1000 cm layers (Fig. S4). Therefore, short-term cultivation of alfalfa pasture was more efficient for carbon accumulation, while long-term cultivation was more favorable for nitrogen accumulation.

The conversion of agricultural land from shallow-rooted annual crops to deep-rooted species such as perennial pasture will impact the regional hydrological cycle, especially groundwater recharging (Acharya et al., 2018). The deep soil water depletion was very difficult to recover, as indicated by previous studies on apple orchards (Zhang et al., 2020) and alfalfa pasture (Ali et al., 2021). In addition, carbon accumulation and water depletion efficiency for carbon accumulation decreased in the middle and late ages of alfalfa, and the advantage of carbon sequestration by alfalfa pasture gradually disappeared. Therefore, in the perspective of water sustainability and carbon accumulation, the long-term alfalfa to long-term annual crop rotation pattern should be converted to short-term alfalfa and short-term annual crop pattern to maintain the length of alfalfa phase in local cropping systems, reduce risks of soil desiccation, and increase soil carbon sequestration.

## Conclusion

We investigated the effects of continuous alfalfa planting on soil water, carbon and nitrogen storage in deep loess. We found soil water depletion by alfalfa mainly occurred in the early ages of alfalfa, and deep soil water storage contributed substantially to alfalfa water use. The SOC storage in all shallow, middle,

and deep soil layers continuously increased from 3 to 6 years after planting, peaked in the 6-year-old alfalfa field, and decreased gradually in the following ages. The soil TN storage only in the shallow layer increased with the age of alfalfa. The deep rooting system of alfalfa derived soil water depletion and SOC and TN increments, especially those in the shallow layer. We suggest short-term alfalfa pasture and short-term annual crop rotation for the deep loess area to increase soil carbon and nitrogen stocks while reducing the risk of soil desiccation.

## Declarations

### Acknowledgments

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### Author contribution

ZW designed the study. LW and AG set up and conducted the field experiment, performed laboratory works and data statistical analyses. ZW and LW drafted the manuscript with suggestions from all the co-authors.

### Data availability

We confirm that, should the manuscript be accepted, the data supporting the results will be available by request from the corresponding author.

### Conflicts of interest/Competing interest

The authors declare that they have no conflict of interest or competing interest.

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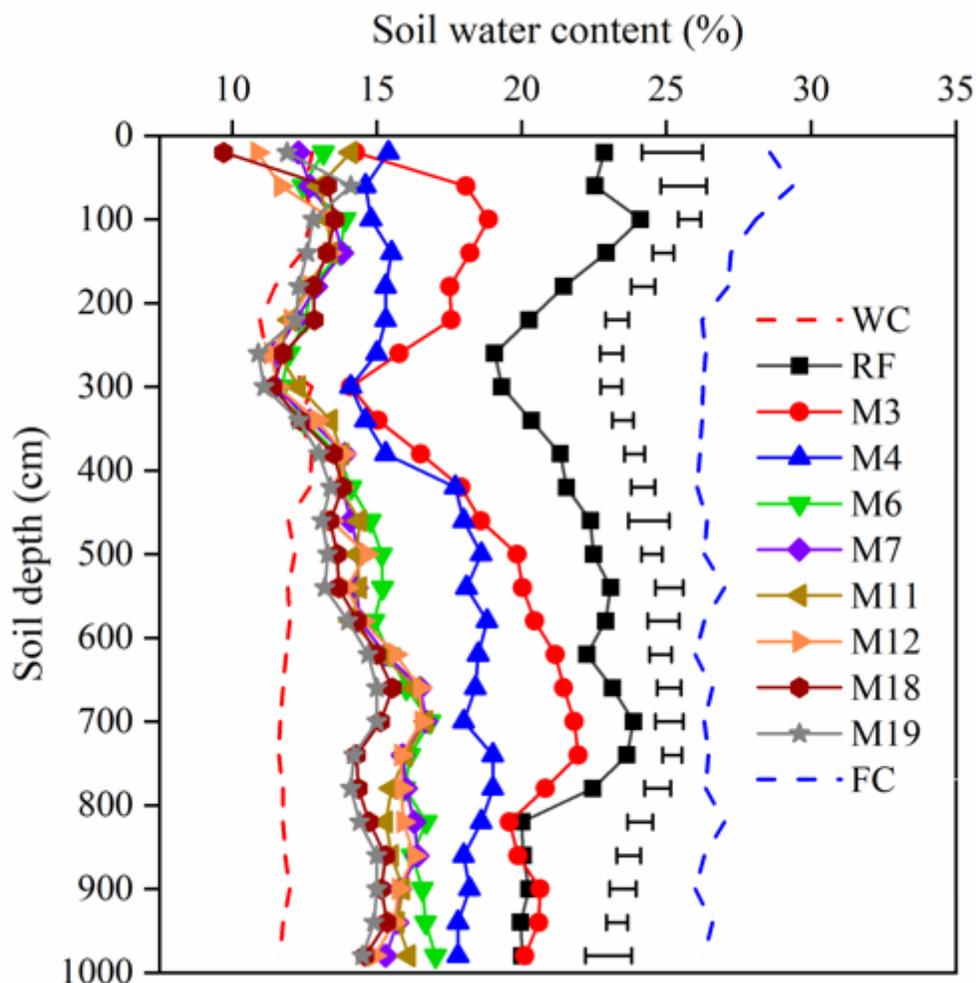
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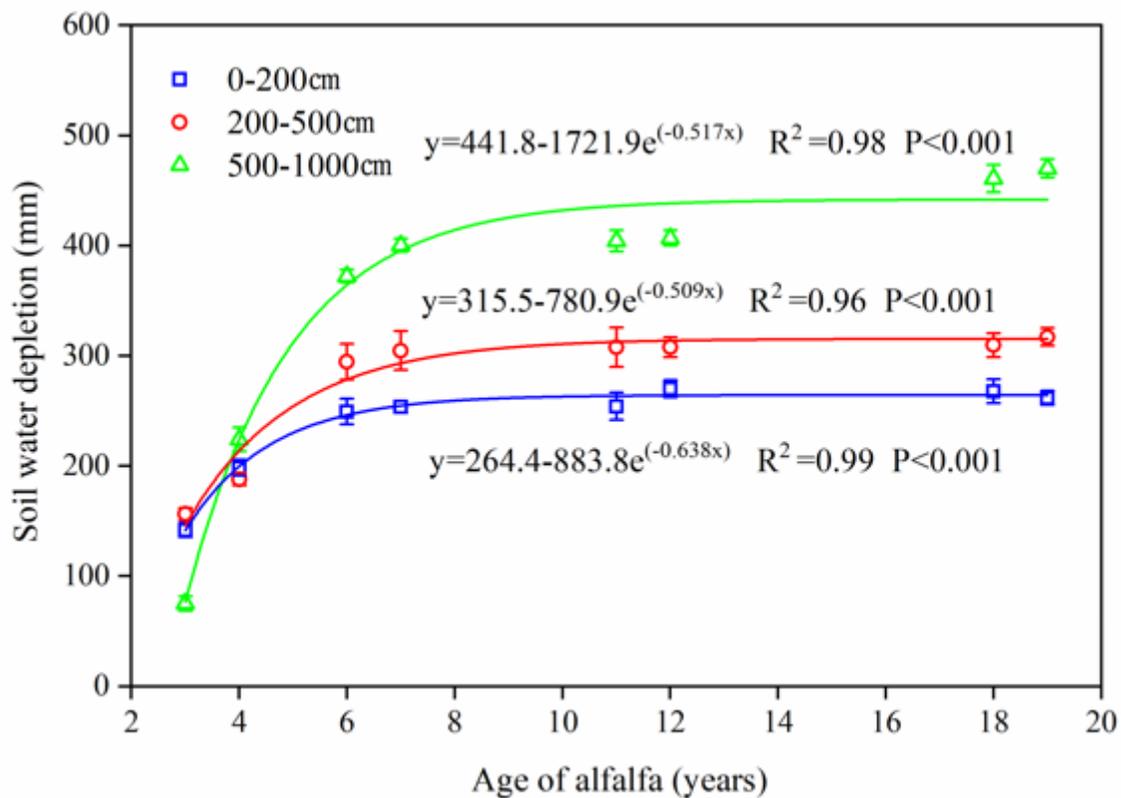
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## Figures



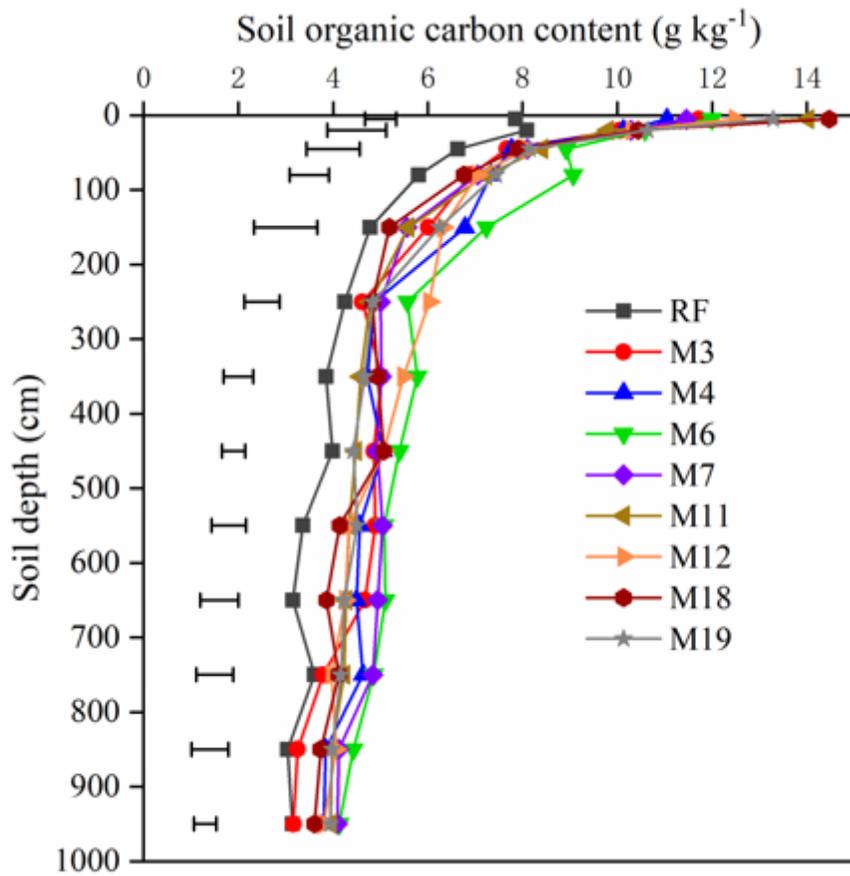
**Figure 1**

Soil water content in the 0–1000 cm profile in different aged alfalfa fields as compared with reference annual crop field. RF refers to the annual crop field; M3, M4, M6, M7, M11, M12, M18, and M19 represent 3-, 4-, 6-, 7-, 11-, 12-, 18-, and 19-year-old alfalfa fields, respectively. The error bars are the LSDs at  $P=0.05$ .



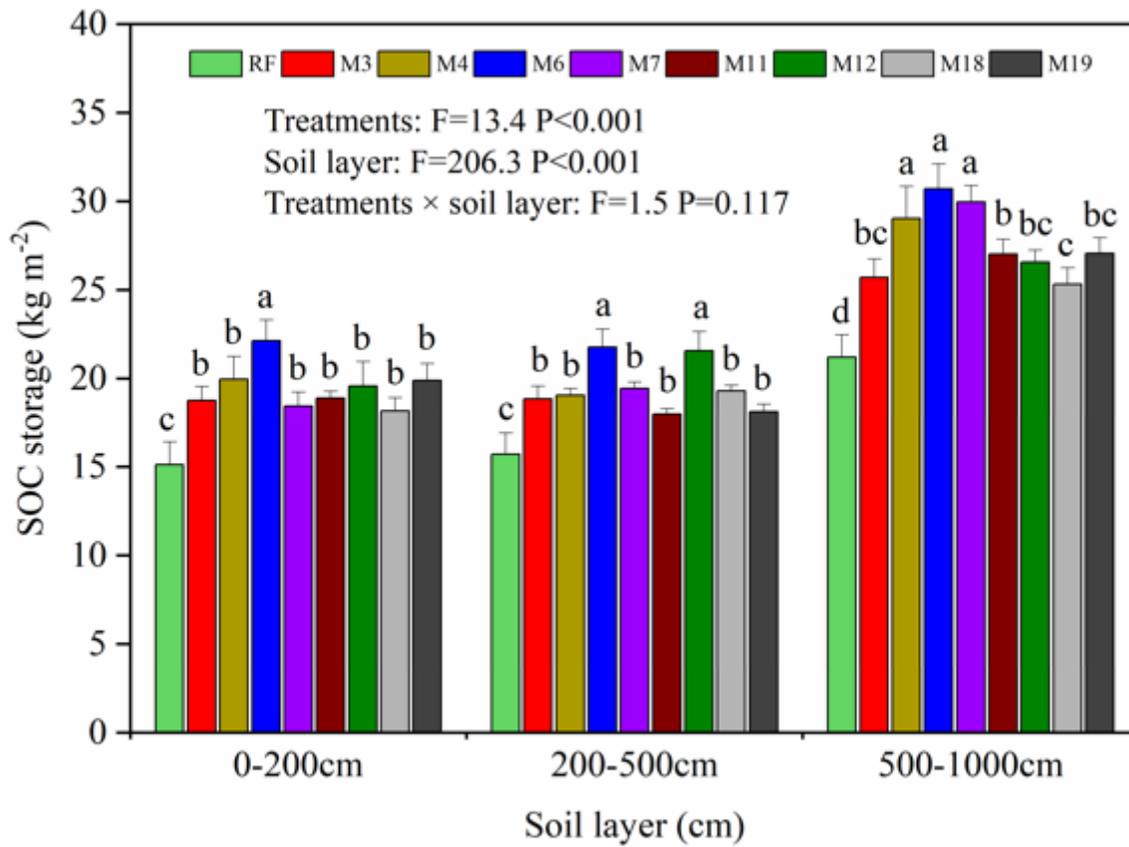
**Figure 2**

Soil water depletion by alfalfa from shallow (0-200 cm), middle (200-500 cm), and deep (500-1000 cm) soil layers and the regression line between soil water depletion and age of alfalfa. The error bars represent standard deviations.



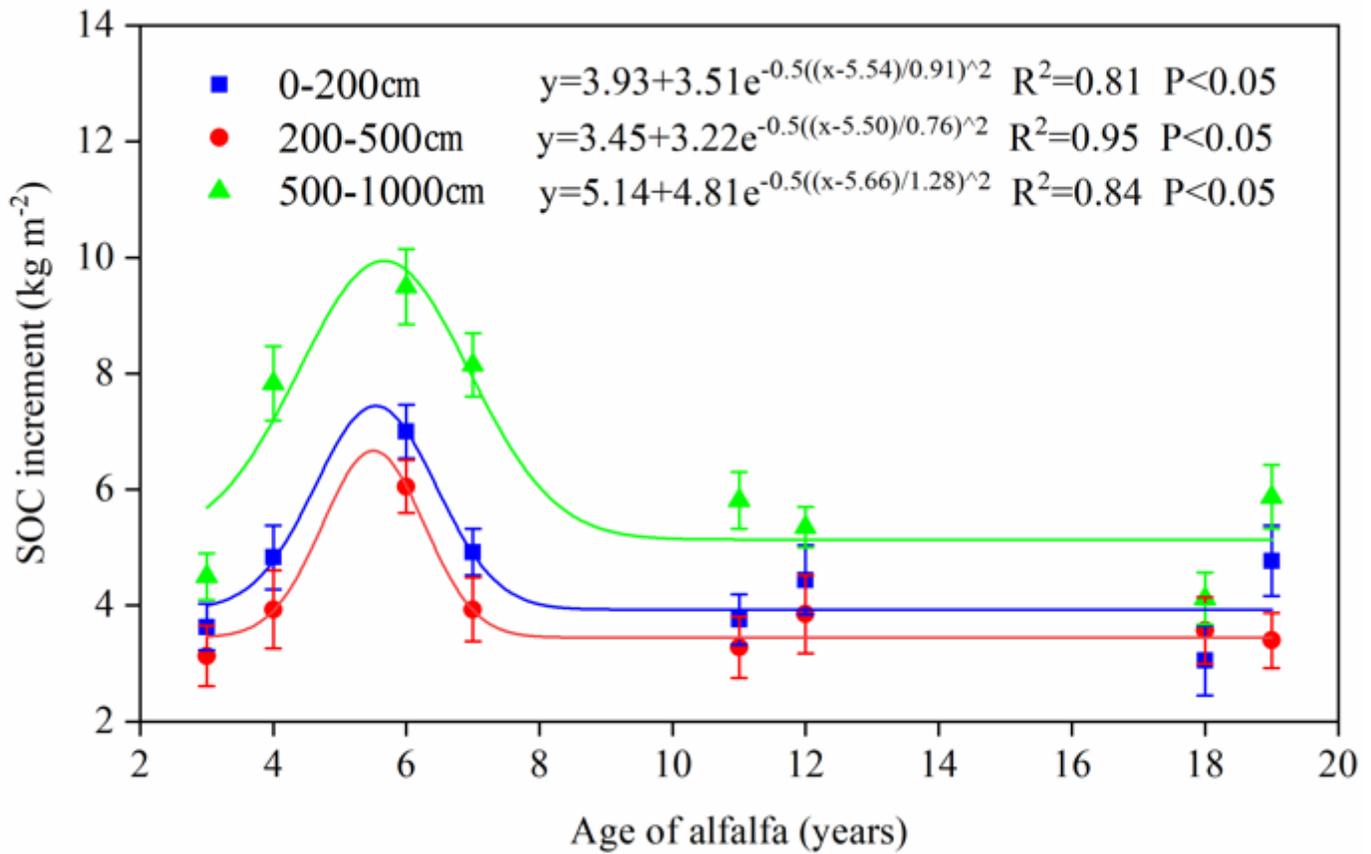
**Figure 3**

Soil organic carbon content in the 0-1000 cm profile in different aged alfalfa fields compared with reference annual crop field. RF refers to the annual crop field; M3, M4, M6, M7, M11, M12, M18, and M19 represent 3-, 4-, 6-, 7-, 11-, 12-, 18-, and 19-year-old alfalfa fields, respectively. The error bars are the LSDs at P= 0.05.



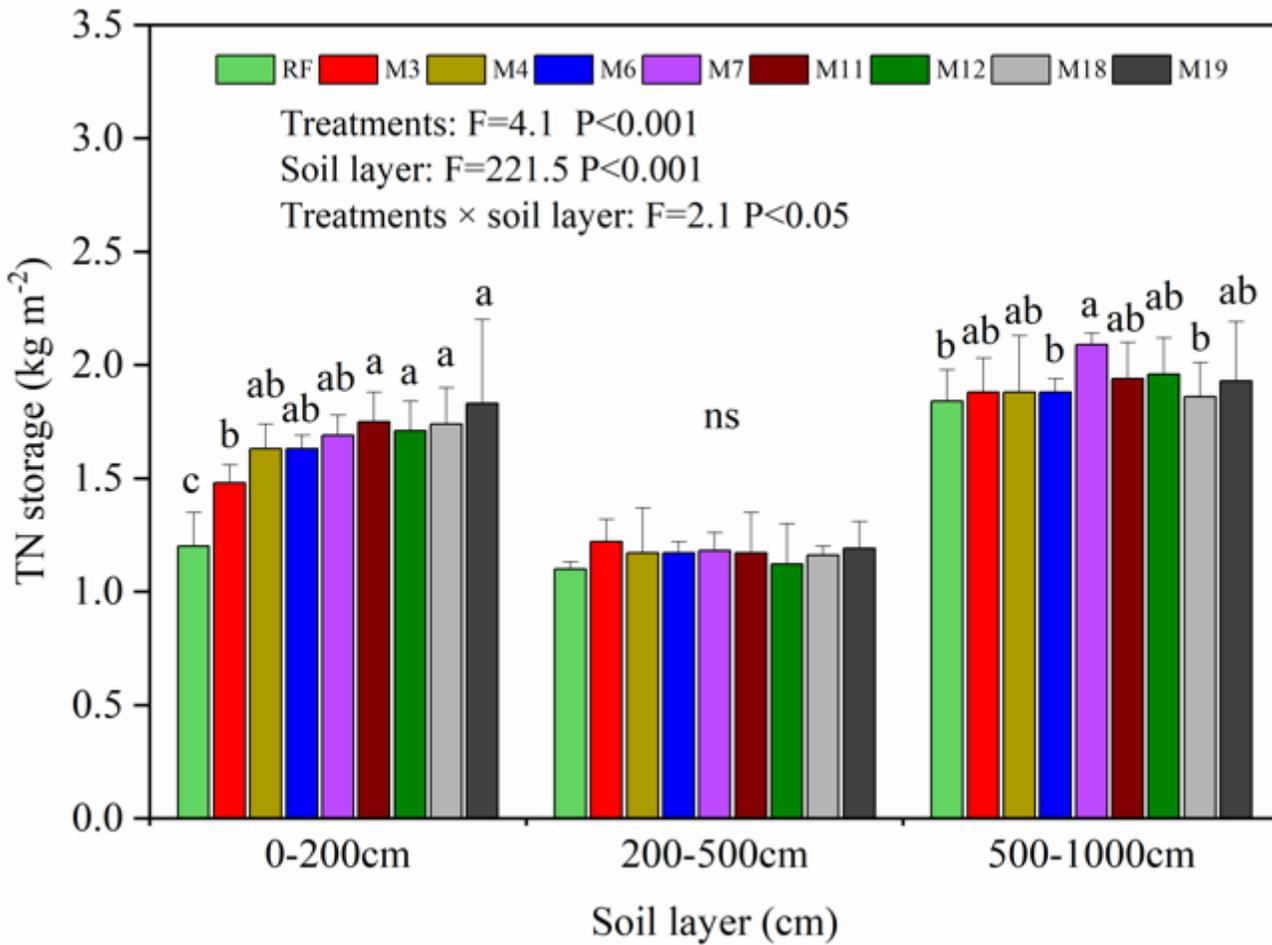
**Figure 4**

Soil organic carbon (SOC) storage in the shallow (0-200 cm), middle (200-500 cm), and deep (500-1000 cm) soil layers in different aged alfalfa fields and annual crops field. RF refers to the annual crop field; M3, M4, M6, M7, M11, M12, M18, and M19 represent 3-, 4-, 6-, 7-, 11-, 12-, 18-, and 19-year-old alfalfa fields, respectively. The error bars represent standard deviations.



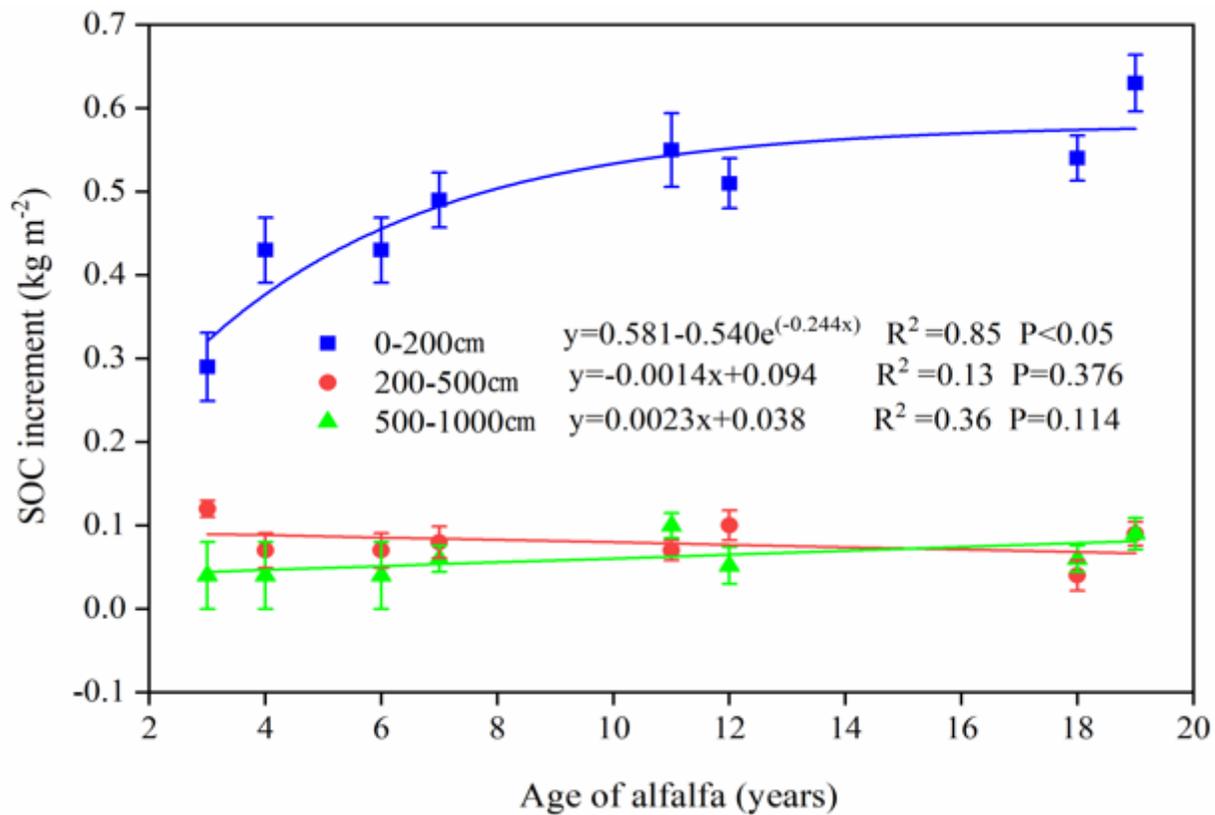
**Figure 5**

The changing trend of soil organic carbon storage increment in shallow (0-200 cm), middle (200-500 cm), and deep (500-1000 cm) soil layers in alfalfa fields as changed with the age of alfalfa. The error bars represent standard deviations.



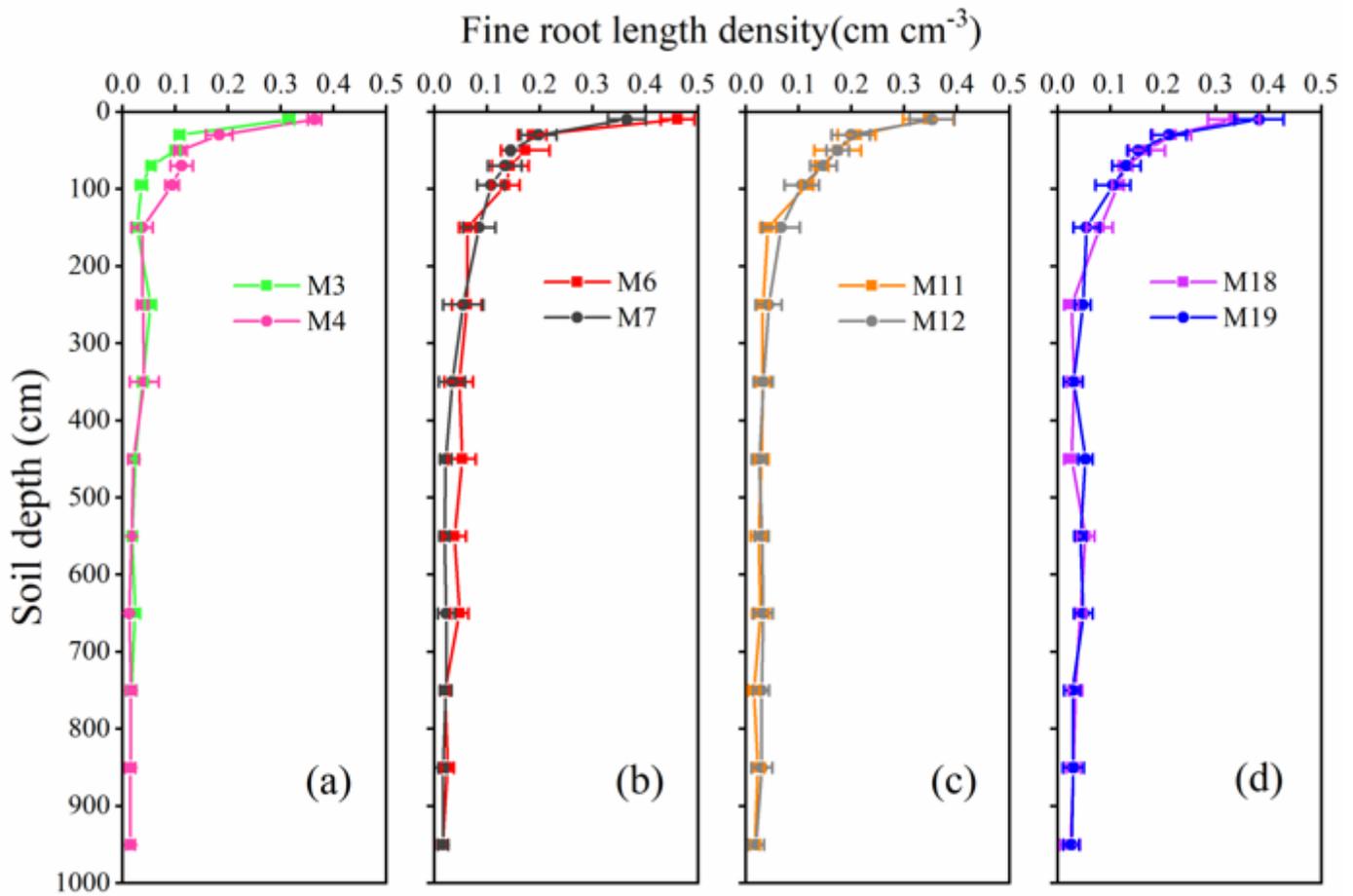
**Figure 6**

Soil total nitrogen (TN) storage in shallow (0-200 cm), middle (200-500 cm), and deep (500-1000 cm) soil layers in different aged alfalfa fields and annual crops field. RF refers to the annual crop field; M3, M4, M6, M7, M11, M12, M18, and M19 represent 3-, 4-, 6-, 7-, 11-, 12-, 18-, and 19-year-old alfalfa fields, respectively. The error bars represent standard deviations.



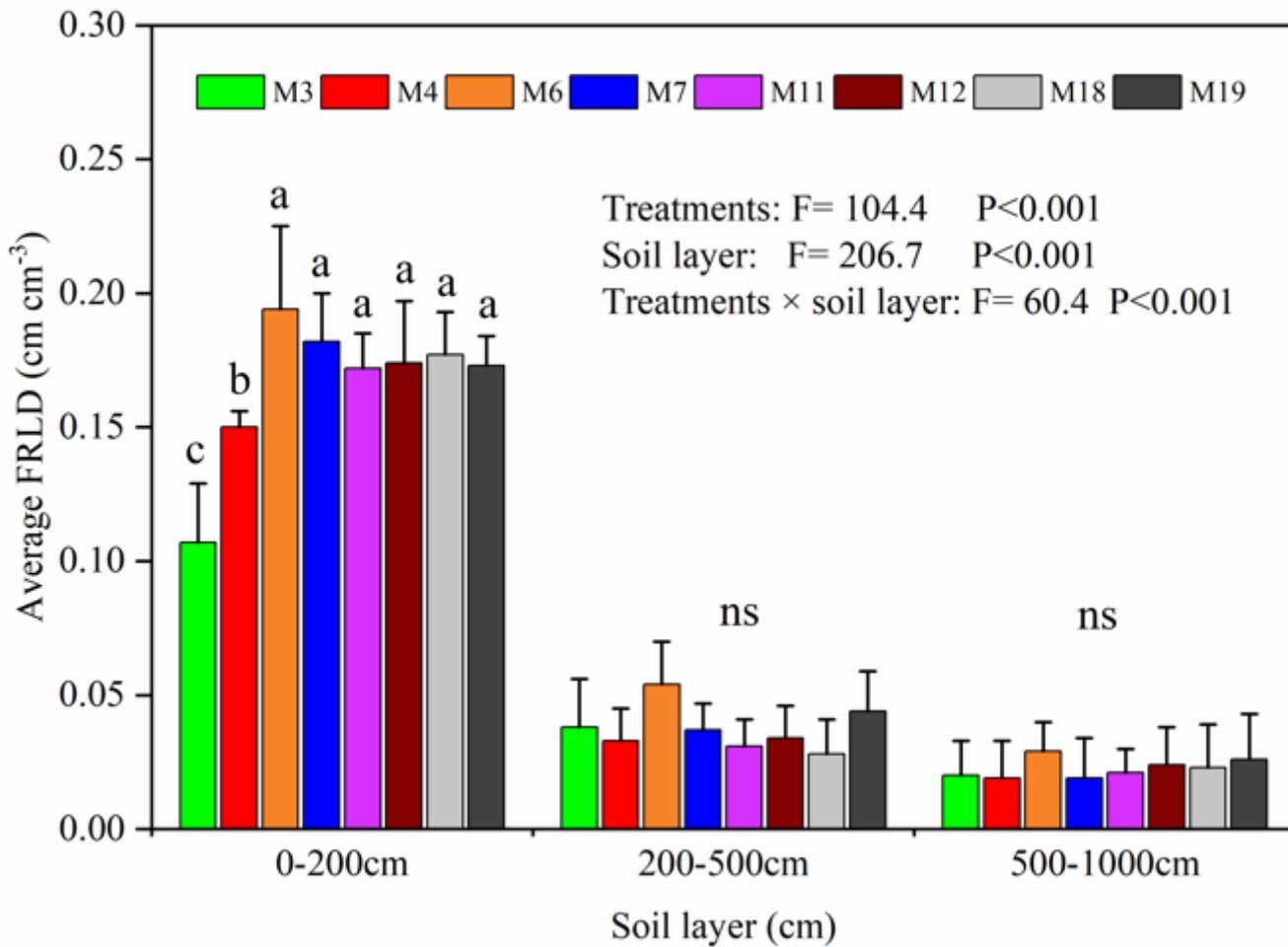
**Figure 7**

Regression analysis of soil total nitrogen (TN) increment in shallow (0-200 cm), middle (200-500 cm), and deep (500-1000 cm) soil layers in alfalfa field as changed with the age of alfalfa. The error bars represent standard deviations.



**Figure 8**

Vertical distributions of fine root length density (FRLD) in 0-1000 cm depth profiles in different aged alfalfa fields. M3, M4, M6, M7, M11, M12, M18, and M19 represent 3-, 4-, 6-, 7-, 11-, 12-, 18-, and 19-year-old alfalfa fields, respectively. The error bars represent standard deviations.



**Figure 9**

Average fine root length density (FRLD) in the shallow (0-200 cm), middle (200-500 cm), and deep (500-1000 cm) soil layers in different aged alfalfa fields. M3, M4, M6, M7, M11, M12, M18, and M19 represent 3-, 4-, 6-, 7-, 11-, 12-, 18-, and 19-year-old alfalfa fields, respectively. The error bars represent standard deviations.

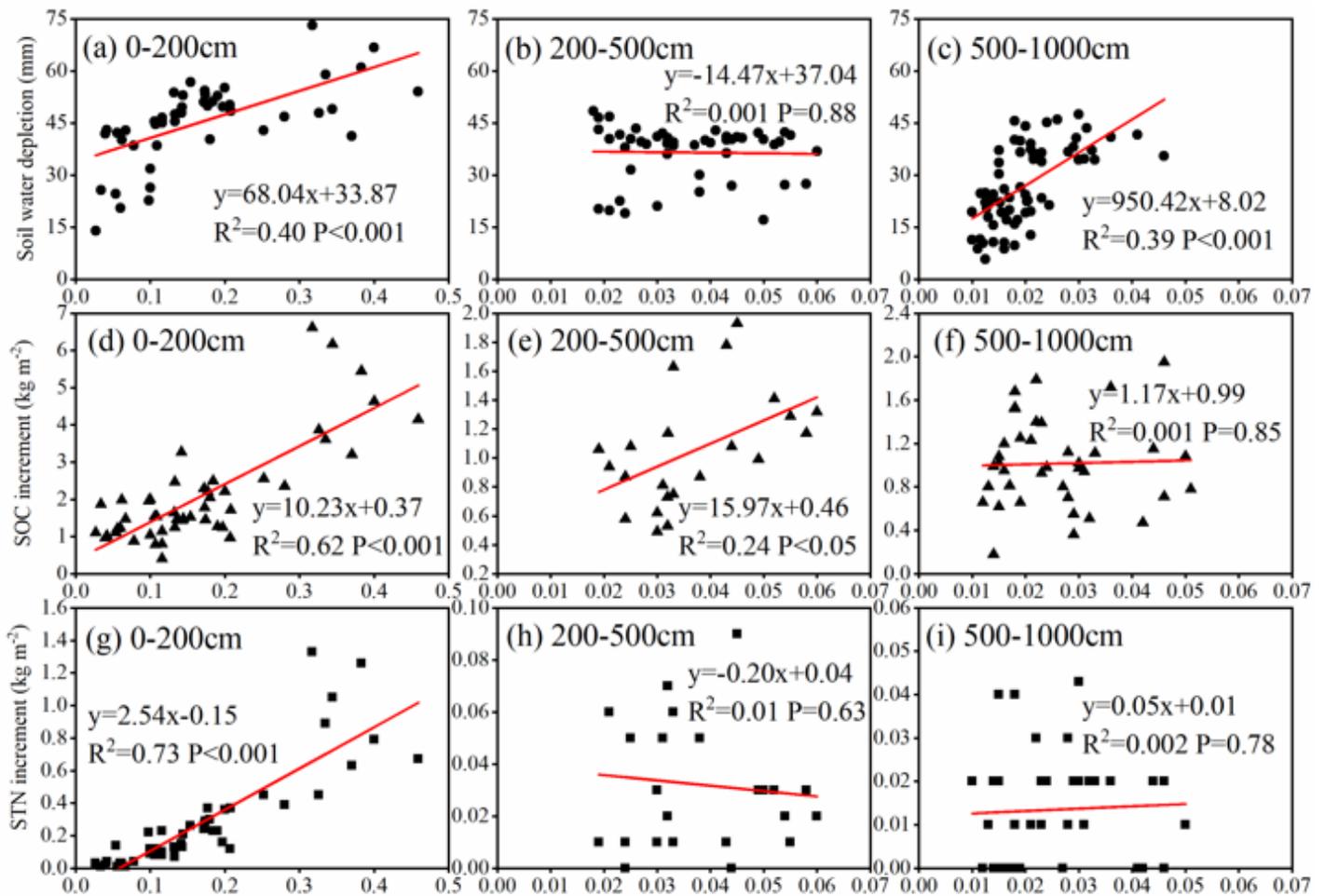


Figure 10

Relationship between fine root length density and soil water depletion, soil organic carbon storage increment, and soil total nitrogen storage increment in the shallow (0-200 cm), middle (200-500 cm), and deep (500-1000 cm) soil layers in different aged alfalfa fields.

## Supplementary Files

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